

CLAIMS

1. A method of analysing a library of polynucleotides, said polynucleotides being contained in cloning vectors having a particular host range, the method comprising (i)
5 selecting cloning vectors in the library which contain a polynucleotide having a particular characteristic, (ii) modifying said selected cloning vectors to allow a transfer and integration of said vectors and/or of the polynucleotide which they contain into a selected host cell, and (iii) analysing the polynucleotides contained in said modified vectors upon transfer of said modified vectors into said selected host cell.
- 10 2. The method of claim 1, wherein the library comprises a plurality of unknown polynucleotides.
3. The method of claim 1 or 2, wherein the library comprises a plurality of
15 environmental DNA fragments.
4. The method of any one of the preceding claims, wherein the cloning vectors of the library are *E. coli* cloning vectors, preferably cosmids, fosmids, P1 or BAC.
- 20 5. The method of any one of the preceding claims, wherein the selected vectors are modified by targeted insertion, into the vector, of a target polynucleotide construct.
6. The method of claim 5, wherein the targeted insertion is performed in a region of the vector distinct from the polynucleotide.
- 25 7. The method of claim 5 or 6, wherein the target polynucleotide construct comprises an origin of transfer functional in the selected host cell.
8. The method of claim 7, wherein the origin of transfer is functional in *E. coli* host cells.

9. The method of claim 8, wherein the origin of transfer is selected from RP4, pTiC58, F, RSF1010, ColE1 and R6K(α).

10. The method of any one of claims 5 to 9, wherein the target polynucleotide construct
5 comprises an integrase functional in the selected host cell.

11. The method of claim 10, wherein the integrase is selected from ϕ C31.

12. The method of any one of claims 6 to 11, wherein the target polynucleotide construct
10 comprises a transcriptional promoter functional in the selected host cell.

13. The method of any one of claims 5 to 12, wherein the target polynucleotide construct
comprises a transposable nucleic acid construct.

14. The method of claim 13, wherein the transposable nucleic acid comprises, flanked by
15 two inverted repeats, the target polynucleotide construct and a marker gene.

15. The method of any one of the preceding claims, wherein the cloning vector
comprises a first marker gene and wherein, in step ii), the cloning vector is modified by:
20 . contacting in vitro, in the presence of a transposase, the selected cloning vectors with a
transposon comprising, flanked by two inverted repeats, the target polynucleotide
construct and a second marker gene distinct from the first marker gene, and
. selecting the cloning vectors which have acquired the second marker gene and which
have lost the first marker gene.

25

16. The method of any one of the preceding claims, wherein, in step (i), the cloning
vectors which contain a polynucleotide having a particular characteristic are selected by
molecular screening.

17. The method of any one of the preceding claims, wherein, in step (iii), the modified
30 cloning vectors are transferred into the selected host cell by conjugative transfer.

18. The method of any one of the preceding claims, wherein, in step (iii), polynucleotides are analysed by determining the phenotype or properties of the host cell upon transfer or expression of the modified vector.

5

19. A method for the identification or cloning of polynucleotides encoding a selected phenotype, the method comprising (i) cloning environmental DNA fragments into *E.coli* cloning vectors to produce a metagenomic library, (ii) identifying or selecting cloning vectors in said library which contain DNA fragments having a particular characteristic of
10 interest, (iii) modifying the identified or selected cloning vectors into shuttle or expression vectors for transfer and integration in a selected host cell, (iv) transferring the modified cloning vectors into said selected host cell and (v) identifying or cloning the DNA fragments contained in said modified cloning vectors which encode said selected phenotype in said selected host cell.

15

20. A transposable nucleic acid construct, wherein said construct comprises an origin of transfer and elements for integration and selection in a selected host cell genome flanked by two inverted repeats.

20

21. A library of polynucleotides, wherein said library comprises a plurality of environmental DNA fragments cloned into cloning vectors, wherein said environmental DNA fragments contain a common molecular characteristic and wherein said cloning vectors are *E. coli* cloning vectors comprising a target polynucleotide construct allowing transfer and integration of the environmental DNA into a selected host cell distinct from

25

E. coli.

22. A polynucleotide sequence comprising all or part of SEQ ID NOs: 1 or 2, or of their complementary strand.

30

23. An oligonucleotide comprising SEQ ID NO: 3 or 4.

BEST AVAILABLE COPY